

AMENDMENTS TO THE SPECIFICATION

In the specification at page 1, after the title and before line 3, please insert the following:

-- RELATED APPLICATIONS

This application is a national stage application (under 35 U.S.C. 371) of PCT/EP2004/003845 filed April 13, 2004 which claims benefit to European application 03008909.8 filed April 16, 2003. –

In the specification at page 33, line 10, please replace the paragraph which starts with “For induced high-level expression” with the following amended paragraph:

For induced high-level expression of the YPR140w gene, a 1146 bp DNA fragment as described in SEQ ID NO: 1, from start to stop codon, was amplified from *S.cerevisiae* W303 genomic DNA by using a 1:1 mixture of Taq and pfu DNA polymerases with the 5' primer, ATGTCTTTTAGGGATGTCCTAGA (SEQ ID NO: 36), and the 3' primer, TCAATCATCCTTACCCTTTGGTT (SEQ ID NO: 37). The resulting PCR product was gel purified, incubated with Taq polymerase and TA-cloned into the pCR2.1-TOPO cloning vector (Invitrogen). The YPR140w gene was then excised from the cloning vector by EcoRI digestion and cloned into the EcoRI behind the strong inducible GAL1 promoter in the multicopy plasmid pYES2 (Invitrogen), thus generating the plasmid pAN3. The wt yeast strain By4742 (MAT α his3 Δ 1, leu2 Δ 0, lys2 Δ 0, ura3 Δ 0), was transformed with pAN3 and pYES2 using standard protocol. Overnight precultures of the two yeast transformants, wt yeast containing pAN3 or pYES2, were grown in 15 ml of synthetic liquid media lacking uracil and supplemented with 2% Glucose (vol/vol). All yeast cultures were grown on rotary shaking at 30°C. The precultures were used to inoculate sixty ml of synthetic liquid media lacking uracil and supplemented with 3% glycerol and 3% lactate to a start OD600 of 0.2. The sixty ml cultures were grown for forty-eight hours (OD600 about 1.5) and then the GAL1 over expression promoter was induced by the addition of galactose to a final concentration of 2% (vol/vol) and the cultures were shaken for a further 6 hours at 30°C. Cells were harvested (OD600 about 2.5) by centrifugation five minutes at 3000 rpm. Pellets were washed once with water and frozen. Cell pellets were thawed,

resuspended in ice-cold breaking buffer (20mM Tris-HCl pH 7.6, 1mM EDTA, 1µg/ml Aprotinin, 0.7 µg/ml Pepstatin, 0.5 µg/ml Leupeptin) to 1,5 ml and added to two ml screw cap tubes containing 1 ml of acid washed glass beads (diameter 0.45-0.5 mm). The tubes were shaken at 4oC for 3 minutes in a Minibeadbeater-8 (Tectum lab and then centrifuged for 10 minutes at 3000 rpm. The supernatant was harvested and stored at -70 oC. Protein concentrations were deter-mined using the BCA-method (Pierce) with BSA as protein standard. Enzymatic assays were run for ten minutes at 30oC. The samples (100µg of protein) were diluted to 50 µl with water and the assay was started by adding 50µl of assay mixture (50 mM Tris-HCl pH 7.5, 8 mM NaF, 4 mM MgCl₂, 0.4 mM 10000dpm/nmol [14C]-G-3-P, 4 mg/ml BSA, 0.2 mM 16:0-CoA, 2 mM DTT). The assays were stopped by adding 400 µl of methanol/chloroform/ glacial acetic acid (50:50:1) and 100 µl of water followed by thoroughly shaking and centrifugation for twenty seconds at 13000 rpm. The bottom chloroform layer was removed and transferred into scintillation vials containing 4 ml of scintillation fluid (ethanol/ethanol 2:1, 0.4% 2(1-butylphenol)-5-(4-biphenyl)-1, 3, 4-oxa-dizole). Radioactivity counting was carried out in a LKB Wallace 1290 Rackbeta Liquid scintillation counter.

In the specification at page 35, line 5, please replace the paragraph which starts with “For induced high-level expression” with the following amended paragraph:

For induced high-level expression of the YPR140w gene, a 1146 bp DNA frag-ment as described in SEQ ID NO: 1, from start to stop codons, was amplified from *S. cerevisiae* W303 genomic DNA by using a 1:1 mixture of Taq and pfu DNA poly-merases with the 5' primer, ATGTCTTTTAGGGATGTCCTAGA (SEQ ID NO: 36), and the 3' primer, TCAATCATCCTTACCCTTTGGTT (SEQ ID NO: 37). The resulting PCR product was gel purified, incubated with Taq polymerase and TA-cloned into the pCR2.1-TOPO cloning vector (Invitrogen). The YPR140w gene was then excised from the cloning vector by EcoRI digestion and cloned into the EcoRI behind the strong inducible GAL1 promotor in the multicopy plasmid pYES2 (Invitrogen), thus generating the plasmid pAN3. The wild type yeast strain By4742 (MATa his3Δ1, leu2 Δ0, lys2 Δ0, ura3 Δ0), transformed with the pAN3 was cultivated at 30°C on a rotary shaker in synthetic medium (Sherman, F. et al., (1986) Laboratory Course Manual for Methods in Yeast Genetics, Cold Spring Harbor Lab. Press, Plainview, NY.) lacking uracil and

supplemented with 2% (vol/vol) glycerol and 2% (vol/vol) ethanol. The GAL1 promoter was induced after 24 hours of growth by the addition of 2% (wt/vol) final concentration of galactose. Cells were harvested after an additional 24 or 28 hours of growth. Wild type cells By4742, transformed with the empty vector (pYES2) and cultivated under identical conditions, were used as a control.

In the specification at page 38, line 10, please replace the paragraph which starts with "To identify possible homologues" with the following amended paragraph:

To identify possible homologues to the YPR140w yeast gene SEQ ID NO: 1, the NCBI database were searched for related plant sequences. From these BLAST searches two protein sequences in *Arabidopsis thaliana* were identified with 31,6 % (At140.1) and 26.3% (At140.2) sequence identity to the deduced amino acid sequence of the open reading frame YPR140w using the GAP programme. From an EST database the full-length sequences of the corresponding cDNAs were identified and named At140.1 and At140.2. The *Arabidopsis* At140.1 and At140.2 genes were then PCR amplified from an *Arabidopsis* seedling cDNA library. The At140.1 gene was amplified from the cDNA by Pfu Turbo DNA polymerase (Stratagene, USA) with the use of the primers At140.1-S1: GTCGGTCTTTCTAACTGAATC (SEQ ID NO: 38) and At140.1-A1: CCTGTGGGACTTAAACCTCA (SEQ ID NO: 39). The PCR product was diluted 10 times and used as a template in a second PCR with internal primers At140.1-S2: CAGAATGGGAATTCATTTTG (SEQ ID NO: 40) and At140.1-A2: CTAACGGGAGTTTAACTTGCA (SEQ ID NO: 41). Similarly, the At140.2 gene was amplified from the cDNA by using the primers At140.2-S1: CTGGTCTCGTTTCTAATTG (SEQ ID NO: 42) and At140.2-A1: CATGGCGAATCTAAACCGGAAC (SEQ ID NO: 43). The resulting DNA fragments were purified and cloned into pCR-Blunt II-TOPO (Invitrogen life technologies, USA), generating pCR-At140.1 and pCR-At140.2, respectively. The genes were verified by sequencing, see SEQ ID NO: 3 and SEQ ID NO: 5.